Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 7137–7141, December 1983
Biochemistry

Structure and expression of the *dnaQ* mutator and the RNase H genes of *Escherichia coli*: Overlap of the promoter regions

(nucleotide sequence/down-promoter mutation/fused protein/*mutD* gene)

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Communicated by Arthur Kornberg, August 19, 1983

ABSTRACT **A 1.6-kilobase-pair DNA fragment derived from the *Escherichia coli* chromosome was analyzed by Tn*3* transposon insertion and deletion mapping to locate a mutator gene, *dnaQ* (*mutD*), and the *rnh* gene that codes for RNase H. When a strong promoter, P_L of λ phage, was placed at the right- and left-side of the cloned DNA fragment, the dnaQ protein and RNase H, respectively were overproduced. These results suggested that the two genes are transcribed in opposite directions and that their promoters are located in a narrow region between the genes. Nucleotide sequence analysis confirmed this and further revealed that transcriptional and translational initiation signals for the two genes overlap. From the sequence data it was deduced that the dnaQ protein and RNase H consist of 243 and 155 triplets and have molecular weights of 27,500 and 17,500, respectively. *dnaQ81* amber mutant showed two codon alterations, CAG(glutamine-195)→TAG(amber) and ACA(threonine-193)→ATA(isoleucine). The *dnaQ–lacZ* and the *rnh–lacZ* fused genes were constructed and hybrid proteins with β-galactosidase activity were produced. From β-galactosidase levels it was estimated that the promoter for *dnaQ* is 5 times more active than that for *rnh*.**

A conditional lethal mutator mutant *dnaQ49* exhibits defective DNA synthesis at restrictive temperature and a strong mutator activity at permissive temperature (1). The properties of the mutant suggested that the *dnaQ* gene product might play a role in the control of fidelity of DNA replication. Recent evidence has shown that DNA polymerase III from *dnaQ49* mutant is defective in 3′→5′ exonuclease activity and that the *dnaQ* gene product may be the ε-subunit of polymerase III holoenzyme (2). *mutD5*, which is also a strong mutator but does not cause temperature-sensitive growth or defective DNA synthesis in cells carrying the mutation (3), was shown to be an allele of the same gene (4) and to be defective in the polymerase III-associated exonuclease activity (2). In this paper, we use the gene symbol *dnaQ* rather than *mutD* because of possible involvement of the gene product in DNA replication.

By *in vitro* recombination we have constructed hybrid plasmids capable of complementing *dnaQ49* mutation (5). The $dnaQ^+$ plasmids contained a 1.6-kilobase-pair (kbp) DNA fragment derived from the *Escherichia coli* chromosome that codes for two proteins, the dnaQ protein and RNase H. It has been shown that RNase H, an enzyme that specifically degrades RNA of DNA·RNA hybrids (6), may play an important role in DNA replication (ref. 7; unpublished results).

To elucidate the structure and expression of the *dnaQ* gene and the structural gene for RNase H (*rnh* gene), we have determined the nucleotide sequence of the DNA containing the genes. We found that the two genes are located only 64 base pairs (bp) apart and transcribe in opposite directions and that their putative transcriptional and translational initiation signals overlap.

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MATERIALS AND METHODS

Bacterial Strains. All bacterial strains used in these experiments are derivatives of *E. coli* K12. Strains KH1113 ($dnaQ^+$), KH1192 ($dnaQ^+$), KH1116 (*dnaQ49*), KH1601 (*dnaQ–rnh*::Tn*3–8*), and KH1602 (*dnaQ–rnh*::Tn*3–9*) were isolated in this laboratory (ref. 1; unpublished results). Strain CSR603 (*uvrA6 recA1 phr-1*) for maxicell experiments was a gift from D. Rupp. Strain CSH26 (*Δlac–pro thi*) was used in experiments for lacZ fused protein.

Plasmids. Plasmid pMM5 ($dnaQ^+$ rnh^+) (5) was used to prepare a 1.6-kbp *Eco*RI fragment (e fragment) for restriction mapping and DNA sequence analysis. Its derivatives, pMM41, pMM42, pMM43, pMM51, pMM52, and pMM53, were constructed by self-ligation after complete or partial digestion of pMM5 with the appropriate restriction enzymes. pKH100 was obtained by inserting the e fragment into the *Eco*RI site of pACYC184 (8). A set of pKH100::Tn*3* plasmids were isolated by the method of Kretschmer and Cohen (9). pKH1 was constructed by introducing an *Eco*RI site downstream of the P_L promoter in pNT26 (pBR322Δ*Eco*RI/*Hin*dIII and λ phage promoter P_L). The e fragment was inserted into the *Eco*RI site of pKH1 to produce pKH11 and pKH16. pNT202, which produces *cI857* repressor and N protein of λ phage, is a derivative of pSC101 (10) and was furnished by M. Imai. The system for high-efficiency expression controlled by the P_L promoter of λ phage was developed by N. Tsurusita, K. Shigesada, and M. Imai. pMC1403 (pBR322Δ*Eco*RI/*Sal* I and lacZ–lacY) (11) used for study of lacZ fused protein was obtained from H. Shinagawa.

***dnaQ* Amber Mutant.** A number of conditional lethal mutants carrying mutations linked to *metD* gene were isolated from a strain harboring *sup126* (temperature-sensitive amber suppressor) (12) by using the method of P1 phage-mediated localized mutagenesis (13). Among them one mutant, designated *dnaQ81*, was found to carry an amber mutation. High mutability of *dnaQ81 sup126* mutant is temperature dependent and the mutator activity is suppressed by *supD*. P1 mapping and complementation studies revealed that the mutation lies in the *dnaQ* gene. λgt*dnaQ81* transducing phage was isolated, and a DNA fragment carrying *dnaQ81* was subcloned into a vector plasmid pBR322, resulting in plasmid pMM81. Details of isolation, characterization, and cloning of the *dnaQ81* mutation will be described elsewhere.

Isolation of Chromosomal Promoter Insertion Mutants. λgt*dnaQ–rnh*::Tn*3–8* and λgt*dnaQ–rnh*::Tn*3–9* were constructed by recloning the Tn*3*-inserted e fragment of pKH100::

Abbreviations: kbp, kilobase pair(s); kDa, kilodalton(s); bp, base pair(s).

Tn3–8 and pKH100::Tn3–9, respectively, into a vector phage λgt-λc (14). They were used to lysogenize KH1192 (*dnaQ*$^+$) cells at the chromosomal *dnaQ–rnh* region. After heat induction of the lysogenized cells, temperature-resistant ampicillin-resistant cells KH1601 and KH1602 were selected.

dnaQ–lacZ and rnh–lacZ Fused Proteins. A 483-bp *Bam*HI fragment from pMM5, carrying both promoters and the beginning of the *dnaQ* and *rnh* genes, as inserted into the *Bam*HI or *Sma* I site of plasmid pMC1403. Based on the DNA sequence data of this region, the coding frame for the *dnaQ* or *rnh* gene was matched with that of *lacZ* gene on pMC1403 DNA. Structures of these plasmids, pMM101 (*dnaQ–lacZ* fusion) and pMM102 (*rnh–lacZ* fusion), are shown in Fig. 5.

Other Methods. Isolation of plasmid DNA, preparation of DNA fragments, restriction mapping, *in vitro* recombination, and transformation were carried out according to ref. 15. DNA sequence determination was done by the chemical degradation method (16). Assay of RNase H activity, determination of mutation frequency, and the procedure for maxicell experiment have been described (1, 5).

RESULTS

Location and Polarity of the *dnaQ* and *rnh* Genes. The *dnaQ* and *rnh* genes of *E. coli* reside on a 1.6-kbp *Eco*RI fragment that is carried by a hybrid plasmid, pMM5 (5). We have introduced transposon Tn3 at various sites of the fragment and also isolated deletion mutants that lacked various lengths of the left- or right-hand region of the fragment (Fig. 1*a*). Each mutant was then assayed for mutator (for the *dnaQ* gene) and RNase H (for the *rnh* gene) activities, and approximate regions that the two genes occupy were determined. The results are summarized in Fig. 1.

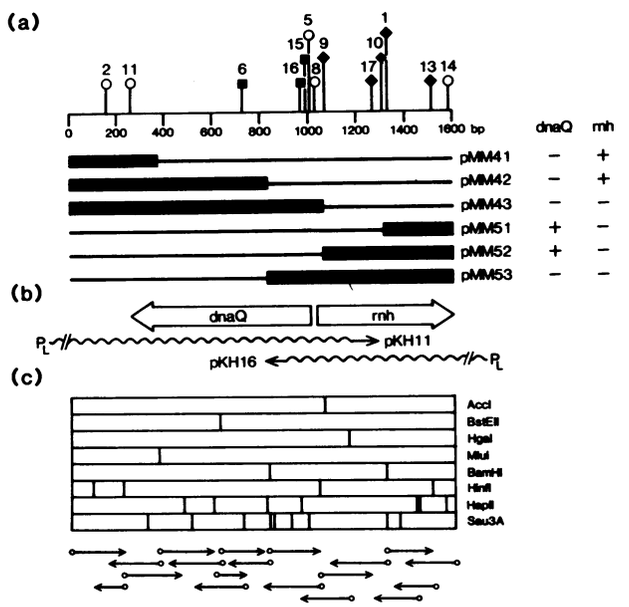

FIG. 1. Physical maps of the *dnaQ–rnh* region. (*a*) Insertion and deletion mutations in the *Eco*RI fragment carrying the *dnaQ* and *rnh* genes. Tn*3* insertion sites were estimated from restriction patterns on 5% polyacrylamide gels. Locations of materials missing in various deletion derivatives of pMM5 are indicated by heavy lines. Inactivation of the *dnaQ* and *rnh* genes by each insertion or deletion mutation was judged by loss of the ability to suppress the mutator activity caused by chromosomal *dnaQ49* mutation or to increase the level of RNase H activity in the cells. Based on this, the Tn*3* insertion mutations were classified into three genotypes, *dnaQ*$^+$ *rnh*$^+$ (○), *dnaQ*$^-$ *rnh*$^+$ (■), and *dnaQ*$^+$ *rnh*$^-$ (◆). The phenotypical changes of deletion derivatives are indicated on the right-hand side for each plasmid. (*b*) Location and the polarity of the *dnaQ* and *rnh* genes. Directions of read-through transcription from P_L promoter on plasmid pKH11 and pKH16 are shown. (*c*) Restriction map and strategy for determining the nucleotide sequence of the *dnaQ–rnh* region. The arrows indicate the direction of sequence analysis and the length of sequences determined in individual experiments.

To determine the direction of transcription of these genes, a strong promoter, P_L of λ phage, was placed at the left- or right-hand side of the cloned DNA fragment, and proteins synthesized by the read-through transcripts were analyzed by polyacrylamide gel electrophoresis. As shown in Fig. 2, cells harboring plasmid pKH11 (P_L–*dnaQ–rnh*) overproduced a 21-kilodalton (kDa) protein after heat induction. Because RNase H activity of these cells was more than 100-fold that of cells harboring plasmid pBR322 and the 21-kDa protein comigrated with RNase H on the gel (data not shown), we concluded that this protein is RNase H and that the *rnh* gene is transcribed rightward on the physical map (Fig. 1*b*). On the other hand, a 27.5-kDa and a 20.5-kDa protein were overproduced in cells harboring pKH16 (P_L–*rnh–dnaQ*). This 27.5-kDa species was considered to be the dnaQ protein, based on the finding that it comigrates with the dnaQ protein on the gel. The 20.5-kDa protein might not be RNase H, but rather a protein coded by some region downstream of the *dnaQ* gene, because there was no increased level of RNase H activity in cells harboring pKH16; the 20.5-kDa protein, but not the 27.5-kDa protein, disappeared when N protein of λ phage, which acts as an antiterminator (17), was absent. Thus, the *dnaQ* and *rnh* genes appear to be transcribed in opposite directions (Fig. 1*b*).

Nucleotide Sequence of the *dnaQ–rnh* Region. The nucleotide sequence of the whole *Eco*RI fragment has been determined by the chemical method (Fig. 3). The strategy for the sequence analysis is shown in Fig. 1*c*, and >80% of the sequence was determined from both strands of the DNA. Two open frames, yielding proteins with molecular weights of 27,500 and 17,500, were detected, which correspond to the *dnaQ* and the *rnh* gene products, respectively. Not only the size but also the location and polarity of the genes are in accord with the data

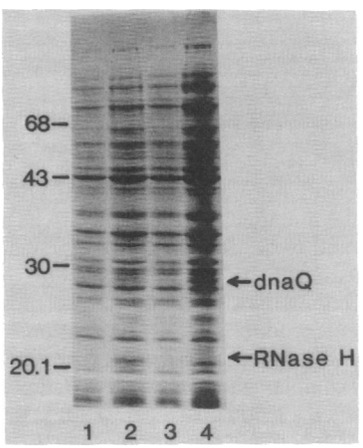

FIG. 2. Overproduction of the dnaQ protein and RNase H. Plasmids pKH11 and pKH16 were used to transform strain HB101, which carries a compatible plasmid, pNT202, that produces thermosensitive *cI857* repressor and N protein of λ phage. These transformants were grown in L broth at 30°C to a density of 2×10^8 cells/ml, and the cultures were shifted to 42°C (0 hour) and incubated for 4 hr. Samples were resolved by electrophoresis in 12.5% polyacrylamide/NaDodSO$_4$ gels, and the gels were stained with Coomassie brilliant blue. Lanes 1 and 2, HB101/pNT202 and pKH11, 0 and 4 hr after heat induction, respectively; lanes 3 and 4, KH101/pNT202 and pKH16, 0 and 4 hr after heat induction, respectively. The position of bovine serum albumin (68 kDa), ovalbumin (43 kDa), bovine erythrocyte carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa), and bovine milk α-lactalbumin (14.4 kDa) are indicated.

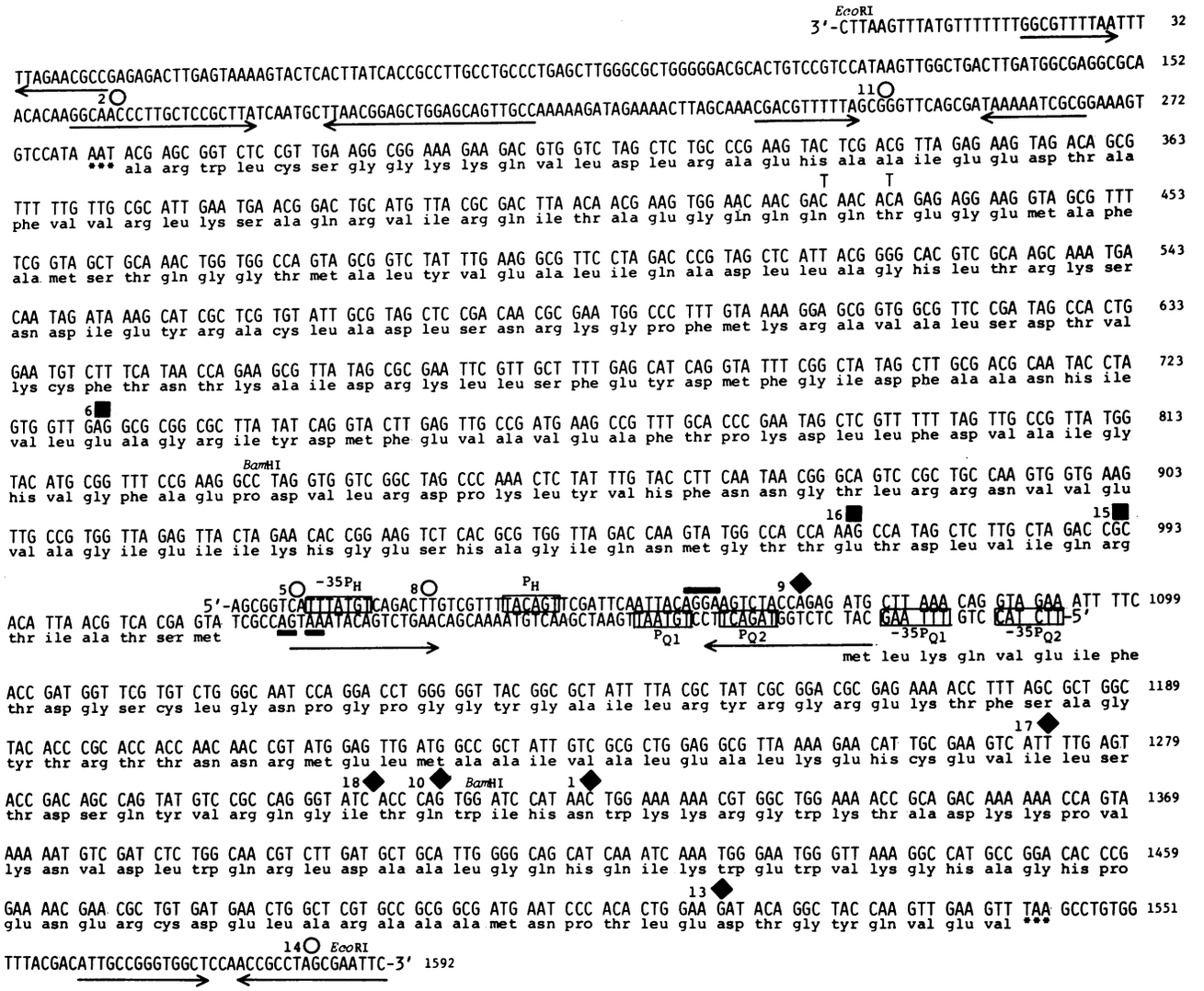

FIG. 3. Nucleotide sequence of the *dnaQ* and *rnh* genes. The nucleotide sequence of the coding strand of the *dnaQ* gene is given 3′ to 5′ and that of the *rnh* gene is given 5′ to 3′. The nucleotides are numbered from the *Eco*RI cleavage site. The restriction sites for *Eco*RI and *Bam*HI are shown. The −10 and −35 sequences for the *dnaQ* (P_Q1, P_Q2, $-35P_Q1$, $-35P_Q2$) and for the *rnh* (P_H, $-35P_H$) are shown enclosed in boxes. Bold lines designate possible ribosome binding sites. The predicted amino acid sequences are shown below the DNA sequences. The regions of dyad symmetry are marked by horizontal arrows. The symbols above the nucleotide sequence represent the approximate positions of the Tn*3* inserts (see the legend to Fig. 1*a*). Base substitutions observed in the *dnaQ81* mutant are shown at positions 429 and 434.

presented above (Fig. 1). Though there are two methionine start codons near the beginning of the *dnaQ* gene (at 1,011 and 1,020 bp), we assume that the ATG codon at 1,011 bp is the initiation site for translation of the *dnaQ* gene because a putative ribosome binding site (18) is located between positions 1,017 and 1,021.

To ascertain that the coding frame assigned for the *dnaQ* is correct, the sequence of a *dnaQ* amber mutant, designated *dnaQ81*, was determined. As shown in Fig. 3, two base changes were found, at positions 429 and 434, resulting in alteration of two codons, CAG(glutamine)→TAG(amber) and ACA(threonine)→ATA(isoleucine), in the frame of the *dnaQ* gene. From this amber mutation site we expect a truncated dnaQ amber protein with a molecular weight of 22,000 to be produced. In a maxicell experiment with *dnaQ81* plasmid we have detected two protein bands, which correspond to the truncated protein and its partial degradation product (data not shown).

Sequences similar to those assigned for the −35 and −10 region of promoter (19–22) were found in front of each gene. In the case of the *dnaQ*, an additional sequence, P_Q2, was detected four bases upstream of P_Q1. It is also noted that the −35 region of the *dnaQ* promoters overlap the beginning of the *rnh* gene.

Down-Promoter Mutations for the *dnaQ* and *rnh* Genes. Effects of Tn*3* insertion mutations on the expression of the two genes were examined by using the maxicell technique (23). The result is shown in Fig. 4, and amounts of the proteins produced were quantified by densitometric scanning of the gel profiles (Table 1). The Tn*3* inserts mapped within each coding region (Fig. 3) abolished a band corresponding to the product of the gene into which Tn*3* was inserted. On the other hand, an insertion mutation, Tn*3*–*8*, which was mapped between the *dnaQ* and the *rnh* genes, decreased the level of expression of both genes. With the Tn*3*–*9* insertion, which is located very near or within the *rnh* coding region, expression of the *dnaQ* gene was somewhat decreased. Similarly, the level of expression of the *rnh* gene was decreased by the Tn*3*–*5* insertion that is located just before the *dnaQ* gene. These results are consistent with the sequence data, which indicate that the transcriptional and translational control signals and also the beginning of the cod-

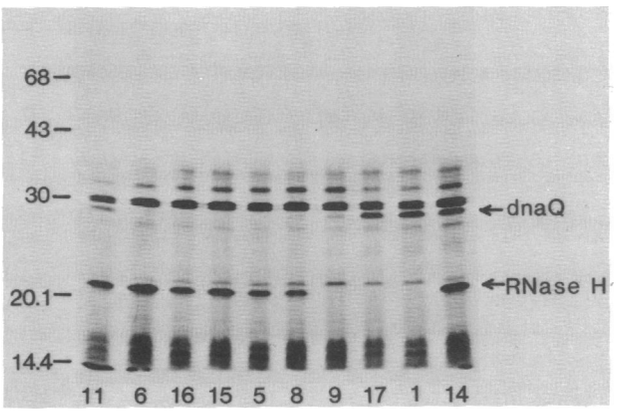

FIG. 4. Effects of Tn*3* insertion mutations on the expression of the *dnaQ* and *rnh* genes. The polypeptides coded for by KH100::Tn*3* plasmids were labeled with [^{35}S]methionine by using the maxicell system and were analyzed by electrophoresis on 12.5% polyacrylamide/NaDodSO$_4$ gel followed by fluorography. The number below each lane corresponds to the Tn*3* insertion mutation number shown in Fig. 1*a*.

ing region for the *dnaQ* and *rnh* genes overlap.

We have transferred these down-promoter mutations from the plasmids to the *E. coli* chromosome and examined the effects of the decreased levels of expression of the *dnaQ* gene on cell growth and fidelity of DNA replication. Strains carrying one of such insertion mutations, Tn*3*–*8* and Tn*3*–*9*, on their chromosome exhibited normal growth even at 44.5°C. However, spontaneous mutation frequencies of these strains were 27 to 720 times higher than that of wild-type strain (Table 2). This implies that not only the quality but also the amount of the dnaQ protein are important for executing accurate DNA replication.

Formation of dnaQ–lacZ and rnh–lacZ Fused Proteins. To obtain further insight into the expression of the *dnaQ* and *rnh* genes *in vivo*, the *lacZ* gene, whose product is readily assayed, has been placed under the control of the *dnaQ* and *rnh* promoters. To do this, a *Bam*HI fragment containing the putative promoter region and the beginning of the two genes was excised from pMM5 and inserted into the *Bam*HI or *Sma* I site of pMC1403 (11) (Fig. 5). If an actual promoter is within the fragment and the correct translational reading frame is maintained according to the nucleotide sequence data, a chimeric protein carrying β-galactosidase activity would be produced. As shown in Table 3, relatively high levels of β-galactosidase activity were found in cells harboring pMM101 (*dnaQ–lacZ* fused gene) or pMM102 (*rnh–lacZ* fused gene). Assuming that the specific activity of β-galactosidase is unchanged by the presence of the fused dnaQ and RNase H polypeptides, we estimate that the promoter for the *dnaQ* is 5 times more active than that for the *rnh*.

Table 1. Expression of the *dnaQ* and *rnh* genes in pKH100::Tn*3* plasmids

Tn*3* insertion	Relative expression efficiency	
	dnaQ	*rnh*
Tn*3*–*11*	1.0	1.0
Tn*3*–*6*	—	1.52
Tn*3*–*16*	—	0.57
Tn*3*–*15*	—	0.62
Tn*3*–*5*	—	0.38
Tn*3*–*8*	0.06	0.26
Tn*3*–*9*	0.27	—
Tn*3*–*17*	1.52	—
Tn*3*–*1*	2.0	—
Tn*3*–*14*	1.0	1.05

Each lane of the fluorographic exposure shown in Fig. 4 was scanned with a densitometric scanner, and the optical densities of peaks corresponding to the dnaQ protein and RNase H were divided by those of the dual peaks of β-lactamase for each lane. Relative expression efficiency refers to the ratio of the value for each pKH100::Tn*3* plasmid to the value for pKH100::Tn*3*–*11*, with 1.0 reflecting equivalence.

Table 2. Mutation frequencies of *dnaQ* down-promoter mutants

Strain	Genotype	Rif^s → Rif^r mutation frequency	Ratio
KH1113	*dnaQ*$^+$	2.5×10^{-8}	1.0
KH1116	*dnaQ49*	1.8×10^{-4}	7,200
KH1601	*dnaQ–rnh*::Tn*3*–*8*	1.8×10^{-5}	720
KH1602	*dnaQ–rnh*::Tn*3*–*9*	6.7×10^{-7}	26.8

Frequency of rifampicin-resistant mutation (Rif^s → Rif^r) is the mean of three experiments. The cells were grown in L broth at 37°C.

DISCUSSION

We have determined the nucleotide sequence of a 1.6-kbp *Eco*RI fragment containing the *dnaQ* and *rnh* genes of *E. coli*. Two open reading frames, which code for a 27.5- and a 17.5-kDa protein, were found in this nucleotide sequence, corresponding to the *dnaQ* and the *rnh* gene, respectively, as judged from their sizes, locations, and polarities. Recently, Kanaya and Crouch (25) reported the DNA sequence of the *rnh* gene, which is exactly the same as the nucleotide sequence from 834 to 1,591 bp shown in Fig. 3. They also indicated that the amino acid sequence of RNase H predicted from the nucleotide sequence is in good agreement with the amino acid composition, the NH_2-terminal sequence, and the molecular weight of highly purified

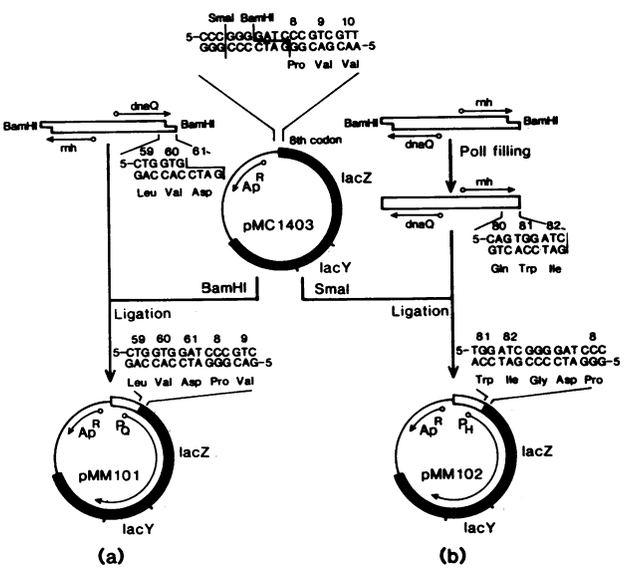

FIG. 5. Construction of plasmids carrying *dnaQ–lacZ* and *rnh–lacZ* fused genes. A DNA fragment containing the *dnaQ* and *rnh* promoter and the beginning of these genes was excised from plasmid pMM5 by *Bam*HI treatment. (*a*) The *Bam*HI fragment was inserted into the *Bam*HI site of pMC1403 to construct pMM101. (*b*) To match the coding frame for the *rnh* gene with that of the *lacZ* gene, cohesive ends of the *Bam*HI fragment were filled in by using the large fragment of *E. coli* DNA polymerase I and then the fragment was inserted into the *Sma* I site of pMC1403. Numbers of amino acid residues of each protein are indicated above triplet codons. The arrows indicate directions of transcription.

Table 3. β-Galactosidase activities of dnaQ–lacZ and rnh–lacZ fused proteins

Plasmid	β-Galactosidase activity, A_{600} units	Fused protein content, molecules per cell
None	<0.1	<1
pMC1403	<0.1	<1
pMM101	1,122	8,800
pMM102	231	1,800

E. coli CSH26 was the host strain. Cells were grown in M9 medium supplemented with 0.1% Casamino acid/thiamine (2 μg/ml)/ampicillin (50 μg/ml) at 37°C. Specific activities of β-galactosidase were determined and contents of fused protein per cell were calculated as described by Miller (24).

RNase H. We previously reported that the dnaQ protein comigrates with the 25-kDa chymotrypsinogen A (5). However, with size markers used in the present experiments (Figs. 2 and 4), the molecular weight of the dnaQ protein on polyacrylamide/NaDodSO$_4$ gels was estimated to be 27,500. This value is close to the value of 28,000, which was given by Cox and Horner for the *mutD* (*dnaQ*) gene product (26). Although the coding frame assigned for the dnaQ protein was confirmed by the sequence data of *dnaQ* amber mutant, more rigorous proof must be obtained from analysis of the protein.

We have presented evidence that the *dnaQ* and *rnh* genes are transcribed in opposite directions and that there is a 64-bp intergenic region between them. Located within this region, from 1,012 to 1,075 bp, are two tandem Pribnow boxes for the *dnaQ* (P_Q1 and P_Q2) and another Pribnow box for *rnh* (P_H) together with proper −35 sequences. A 490-bp *Bam*HI fragment containing this spacer region had promoter activities that start transcription in both directions, as revealed by formation of hybrid proteins on gene fusion. Moreover, all Tn*3* insertions downstream but not upstream of P_Q1 or P_H greatly decreased expression of the *dnaQ* or *rnh* gene. Further analysis of transcripts of these genes would be pertinent, but the facts mentioned above strongly suggest that these putative promoters must act as such *in vivo*. Although oppositely overlapping promoters were previously reported in a plasmid genome (27), in this case promoters were shown to oppositely overlap in the *E. coli* chromosome.

There is a 16-bp inverted repeat within the spacer region. This might be a control region for expression of the *dnaQ* and *rnh* genes, to which a common effector might interact. Although changes in RNase H activity accompanying changes in cell activity have been observed in mammalian cells (28, 29), little is known about the regulation of *dnaQ* and *rnh* gene expression. The plasmids producing the dnaQ–lacZ or rnh–lacZ fused protein under the control of their own promoters would be useful for studying this problem.

The dnaQ protein is considered to control the editing function of the DNA polymerase III, because the polymerase III from *dnaQ49* and *mutD5* mutant strains are defective in 3′→5′ exonuclease activity (2). Genetic evidence for interaction between the dnaQ protein and the α-subunit of polymerase III has also been presented (30). It was reported that the ε-subunit of DNA polymerase III comigrates with 25-kDa chymotrypsinogen A (31) and, hence, Horiuchi *et al.* (5) suggested that the *dnaQ* gene product may be the ε-subunit of polymerase III holoenzyme. Recently, Echols *et al.* (2) provided data supporting this idea. If this is the case, it is supposed that in the *dnaQ* down-promoter mutants some polymerase III molecules might lack the ε-subunit, which would cause error-prone DNA synthesis. It would be of interest to study the role of the dnaQ protein by using the *dnaQ* amber mutant, which has been characterized in the present study.

We are grateful to M. Imai and H. Sugisaki for their helpful suggestion and for supplying bacterial strains, plasmids, and enzymes. This study was supported by grants from the Ministry of Education, Science, and Culture of Japan and by a grant from the Mitsubishi Foundation.

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